

September 12, 2001, which amendments were made to incorporate written text that was removed from the drawings when formal drawings were prepared. The written text which was removed from FIG. 11 was inadvertently added to the description for FIG. 10, and a portion of the written text which was removed from FIG. 12 was inadvertently added to the FIG. 11 description. For the convenience of the Examiner, a marked-up copy of the page incorporating the current amendments to the description of the drawings is enclosed.

Should any fees under 37 C.F.R. §§ 1.16 to 1.21 be required for any reason, the Assistant Commissioner is authorized to deduct said fees from Williams, Morgan & Amerson, P.C. Deposit Account No. 50-0786/4300.014100.

Please date stamp and return the accompanying postcard to evidence receipt of these documents.

Respectfully submitted,



Date: March 3, 2003



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number of eyes examined for each condition was at least 16. *Significantly different ($p < 0.05$) from uninjected.

FIG. 10 shows the number of neovascular nuclei counted per eye section for both the uninjected and AAV-IGF1R Rz1 injected eyes. ~~Helix IV is at least 6 bases in length. The underlined bases can be any RNA tetraloop of the form $5'GNRA^{3'}$ or UUCG, where N is any nucleotide and R is G or A. N can be any ribonucleotide (A, C, G or U) and N' is the complementary nucleotide. Y is a pyrimidine. H is any nucleotide but guanosine (A, C or U). B is any nucleotide but adenosine (G, C or U). V is the complement of B (G, C or A).~~

FIG. 11 shows a schematic illustration of a representative hairpin ribozyme molecule of the present invention (SEQ ID NO:105, SEQ ID NO:106). In particular, FIG. 1² shows a general hammerhead ribozyme structure. ~~The italicized positions are constant. The stem may be any 4 or 5 base double stranded helix with a $5'G-C^{3'}$ base pair at the top of the stem as drawn.~~ Helix IV is at least 6 bases in length. The underlined bases can be any RNA tetraloop of the form $5'GNRA^{3'}$ or UUCG, where N is any nucleotide and R is G or A. N can be any ribonucleotide (A, C, G or & and N' is the complementary nucleotide. Y is a pyrimidine. H is any nucleotide but guanosine (A, C or U). B is any nucleotide but adenosine (G, C or U). V is the complement of B (G, C or A).

FIG. 12 shows a schematic illustration of a representative hammerhead ribozyme molecule of the present invention (SEQ ID NO:107). The sequences of the arms may vary, as shown in Tables 4-8. The italicized positions are constant. The stem may be any 4 or 5 base double stranded helix with a $5'G-C^{3'}$ base pair at the top of the stem as drawn. Underlined nucleotides in loop may be $5'UUCG^{3'}$ or $5'GNRA^{3'}$, where N is any nucleotide and R is a purine nucleotide.

3. BRIEF DESCRIPTION OF THE DRAWINGS

The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present invention. The invention may be better understood by reference to the following description taken in conjunction with the accompanying drawings, in which like reference numerals identify like elements, and in which:

FIG. 1 shows adenosine, acting through its type A_2 receptor, can act to increase oxygen supply via two paths. During acute hypoxia, adenosine acts on smooth muscle cells, resulting in vasodilation (A_{2A}). With chronic ischemia, adenosine acts as an angiogenic agent by exerting a mitogenic effect on microvascular endothelial cells (in HREC, A_{2B} ; see below). It is this latter effect that can be interfered with in an attempt to develop a pharmacological therapy for neovascular diseases. A distinct receptor subtype that mediates solely the mitogenic effect of adenosine would allow the targeting of a selective antagonist against that receptor subtype, without preventing the vasodilation mediated by the A_{2A} receptor;

FIG. 2A shows HREC proliferation after stimulation with NECA alone or in combination with a blocking antibody to VEGF. Open bars are results after 24 hr of exposure; filled bars are results after 48 hr. (*), significantly different from 10 μ M NECA alone for the respective exposure time by ANOVA ($p < 0.05$). Also shown are control cells exposed to VEGF alone or in combination with anti-VEGF to demonstrate the efficacy of the antibody;

FIG. 2B shows VEGF content in conditioned medium from HREC after stimulation with NECA in the presence or absence of sense or antisense oligonucleotides homologous to human A_{2B} adenosine receptor or to human VEGF. Assay duration was 48 hr. A_{2B} antisense treatment reduces the amount of VEGF protein secreted in response to NECA to levels equaling or exceeding the reduction evident by VEGF antisense treatment;

FIG. 3A and **FIG. 3B** show NECA, at the concentrations indicated in the legends, induces a transient activation of ERK/MAPK in HREC that peaks at 5 min and desensitizes by 20 min after exposure. HREC were serum-starved for 24 hr and pre-treated for 20 min with 1 U/mL adenosine deaminase prior to adding NECA. Activated ERK/MAPK was visualized on Western blots by enhanced chemiluminescence using EC10 monoclonal antibody;

FIG. 4A, **FIG. 4B** and **FIG. 4C** show the A_1 -selective agonist CPA stimulates ERK/MAPK phosphorylation in HREC, however the A_{2A} -selective agonist CGS did not activate ERK/MAPK;

FIG. 5 shows HREC were pretreated for 30 min with the MEK inhibitor PD98059 or the PKA inhibitor H-89 and stimulated with NECA for 5 min. PD98059 inhibited ERK activation, while H-89 increased basal ERK activation. H-89 did not block NECA-stimulated ERK activation, suggesting that PKA is not involved in signaling from the adenosine receptor to ERK. The non-selective adenosine receptor antagonist XAC decreased ERK activation by high concentrations of NECA, but modestly increased ERK activation in control conditions and in response to 1 and 10 nM NECA. In contrast, PD98059 did not alter CREB, whereas both H-89 and XAC blocked NECA-induced CREB activation. These data indicate that NECA results in ERK activation independent of the cAMP response;

FIG. 6 shows both Enprofylline and JW V-108 antagonize activation of p42 and p44 ERK/MAP kinase by NECA. HRECs were serum-starved for 24 hr and pre-treated with adenosine deaminase (ADA, 1 U/mL) for 20 min, incubated with the antagonists in the presence of ADA for 10 min. NECA (1 nM-10 μ M, 10 min) was used to activate ERK. ERK activation was analyzed by Western blot using the E10 monoclonal antibody, which recognizes the phosphorylated (active) form of the enzyme;

FIG. 7A, FIG. 7B and **FIG. 7C** show a schematic representation (**FIG. 7A**) of the A_{2B} adenosine receptor ribozyme showing the nucleotide sequence of the recognition arms (SEQ ID NO:1), as well as the complementary sequence of the synthetic target (SEQ ID NO:2). Cleavage of this target by the ribozyme is shown in the autoradiogram (**FIG. 7B**), demonstrating the cleavage kinetics. Band densities of cleaved vs. intact target were plotted as percent cleaved (**FIG. 7C**). The A_{2B} receptor ribozyme cleaves nearly 90% of target in a 1:1 molar ratio by 60 min;

FIG. 8A and **FIG. 8B** show A_{2B} adenosine receptor ribozyme reduces NECA-stimulated VEGF synthesis and cell proliferation in HREC. Cells were stimulated with 10 μ mol/L NECA alone (◆), or NECA plus 1 μ mol/L of either a mixed 37-mer oligoribonucleotide (sham, ■) or A_{2B} ribozyme (▲). Both the amount of VEGF secreted into the medium (top) and the degree of proliferation (bottom) were decreased by the ribozyme, and not by the sham oligonucleotide control; and

FIG. 9 shows adenosine receptor antagonists reduce the degree of retinal neovascularization in the mouse pup model of oxygen-induced retinopathy. Daily IP injections of antagonists (30 mg/Kg body weight) resulted in a 54% to 70% reduction compared to untreated controls. The

number of eyes examined for each condition was at least 16. *Significantly different ($p < 0.05$) from uninjected.

FIG. 10 shows the number of neovascular nuclei counted per eye section for both the uninjected and AAV-IGF1R Rz1 injected eyes.

FIG. 11 shows a schematic illustration of a representative hairpin ribozyme molecule of the present invention (SEQ ID NO:105, SEQ ID NO:106). Helix IV is at least 6 bases in length. The underlined bases can be any RNA tetraloop of the form $5'GNRA^{3'}$ or UUCG, where N is any nucleotide and R is G or A. N can be any ribonucleotide (A, C, G or U) and N' is the complementary nucleotide. Y is a pyrimidine. H is any nucleotide but guanosine (A, C or U). B is any nucleotide but adenosine (G, C or U). V is the complement of B (G, C or A).

FIG. 12 shows a schematic illustration of a representative hammerhead ribozyme molecule of the present invention (SEQ ID NO:107). In particular, FIG. 12 shows a general hammerhead ribozyme structure. The sequences of the arms may vary, as shown in Tables 4-8. The italicized positions are constant. The stem may be any 4 or 5 base double stranded helix with a $5'G-C^{3'}$ base pair at the top of the stem as drawn. Underlined nucleotides in loop may be $5'UUCG^{3'}$ or $5'GNRA^{3'}$, where N is any nucleotide and R is a purine nucleotide.